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Update on bacterial pathogens: virulence and resistance

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The present article is an update of the literature on bacterial pathogens. Recognizing the interest and scientific and public health importance of infections produced by bacterial pathogens with new virulence mechanisms and/or new mechanisms of resistance to antimicrobial agents, a multidisciplinary group of Spanish physicians and microbiologists organized a joint session and revised the most important papers produced in the field during 2006. Each article was analyzed and discussed by one of the members of the panel. This paper focus on a variety of diseases that pose major clinical and public health challenges today; and include infections produced by community-acquired methicillin-resistant *Staphylococcus aureus* and *S. aureus* small colony variants, infections produced by multiply resistant coagulase-negative staphylococci, pneumococcal infections, human listeriosis, meningococcal disease, *Haemophilus influenzae*, pertussis, *Escherichia coli*, ESBL-producing organisms, and infections due to non-fermenters. After a review of the state of the art, papers selected in this field are discussed.

Key words: **Bacterial infections. Resistance to antimicrobials. Community-acquired-MRSA. *S. aureus* small colony variants. Resistance to linezolid. Pneumococcal vaccines. *Listeria monocytogenes*. *Neisseria meningitidis*. *Haemophilus influenzae*. *Bordetella pertussis*. *Escherichia coli*. *Klebsiella pneumoniae*. ESBL producers. *Pseudomonas aeruginosa*. *Acinetobacter baumannii*.**

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Actualización en patógenos bacterianos: virulencia y resistencia

El presente artículo recoge una actualización bibliográfica de patógenos bacterianos. Dado el interés científico y la importancia que tienen para la salud pública las infecciones producidas por patógenos bacterianos con nuevos mecanismos de virulencia y/o nuevos mecanismos de resistencia a los antimicrobianos, un grupo multidisciplinario de microbiólogos y clínicos españoles, con experiencia en enfermedades infecciosas, organizó una reunión en la que se revisaron los artículos más importantes en este campo, publicados en 2006. El contenido de cada uno de los artículos seleccionados fue expuesto y discutido por uno de los miembros del grupo. Este artículo revisa algunas de las enfermedades infecciosas bacterianas que suponen hoy en día algunos de los principales retos para la salud pública e incluye las infecciones producidas por *Staphylococcus aureus* resistente a meticilina de adquisición comunitaria, las producidas por variantes de colonia pequeña de *S. aureus*, las relacionadas con estafilococos coagulasa negativa multirresistentes, la infección neumocócica, la listeriosis humana, la infección meningocócica, la tos ferina, las infecciones por *Haemophilus influenzae*, la diseminación de las bacterias productoras de BLEE, y las infecciones por bacilos gramnegativos no fermentadores. Tras la revisión de la situación actual, se discuten y comentan diferentes artículos relacionados con estos aspectos.

Palabras clave: **Infecciones bacterianas. Resistencia a antibióticos. SARM de adquisición comunitaria. Variantes de colonia pequeña de *Staphylococcus aureus*. Resistencia a linezolid. Vacunas neumocócicas. *Listeria monocytogenes*. *Neisseria meningitidis*. *Haemophilus influenzae*. *Bordetella pertussis*. *Escherichia coli*. *Klebsiella pneumoniae*. Productores de BLEE. *Pseudomonas aeruginosa*. *Acinetobacter baumannii*.**

State of the art (Dr. E. Cercenado, Dr. J. Garau)

Although predictions during the 20th century indicated that the incidence of infectious diseases would diminish as a result of improvements in sanitation and by the introduction of many vaccines and antibiotics, at the beginning of the 21st century the rates of infections produced by new pathogens or by reemerging microorganisms possessing new virulence or resistance phenotypes is increasing, threatening the overall human health[1-5]. Over the last 100 years we have moved from the pre-antibiotic era through the antibiotic era into the era of emerging infectious diseases. The discovery of new infectious diseases and the steady increase in the number of microorganisms that are resistant to multiple antimicrobial agents have altered the practice of medicine within the hospital, and are affecting the management of infections in the ambulatory care setting[6,7].

It is in this scenario where *community-acquired methicillin-resistant Staphylococcus aureus* (CA-MRSA) has emerged as the most common pathogen isolated from patients with skin and soft-tissue infections attending to the emergency departments in many United States and Australian cities[8,9], and at present, its incidence is increasing in other parts of the world[10,11]. Despite the growing prevalence of MRSA in hospitals, these strains have been uncommon in the community. In many circumstances MRSA escape into the community when patients still harbouring the organisms are discharged or when hospital personnel go from work to home. These strains can be associated with infections that begin in the community, but the isolates are hospital-associated. However, there is now an increasing number of reports of MRSA infections in the community that have not escaped from the hospitals, and no longer can MRSA be considered as an exclusively nosocomial pathogen. There is molecular evidence that strains of MRSA have also evolved in the community, are well adapted to survive there, and are causing an epidemic outside the hospitals[12,13]. CA-MRSA infections refer to MRSA infections in patients lacking established MRSA risk factors and without a previous history of MRSA infection or colonization: they do not have a medical history in the past year of hospitalization, healthcare-related admission (to a nursing home, skilled nursing facility or hospice), dialysis, surgery, or implantation of a permanent indwelling catheter or other medical devices[8,14]. CA-MRSA tends to cause infections that occur in clusters or small outbreaks that affect otherwise healthy and unique populations such as children and young adults, Australian Aborigines, Native Americans, Alaskan Natives, prisoners, military recruits, men who have sex with men, college athletes and players competing in contact sports. Recent antimicrobial exposure is less likely to be reported as a risk for MRSA infections in the community that in health care settings. Most infections are mild and limited to skin and soft tissues, though rapidly fatal invasive infections such as necrotizing fasciitis and overwhelming pneumonia and sepsis accompanied by the Waterhouse-Friderichsen syndrome do occur and may serve as "sentinels" that first bring a more widespread community problem to the attention of public health personnel[15-20]. Because skin infections caused by these organisms often have necrotic centers, the disease can be misdiagnosed as a "spider bite". Similar to the epidemiology of methicillin-susceptible *S. aureus*, CA-MRSA infections occur in children in different regions of the United States and throughout the world[11,21-24]. Although minor skin and soft-tissue infections predominate, life-threatening invasive disease and death can result. In children, CA-MRSA can cause septic thrombophlebitis of the extremities and a "pelvic syndrome" consisting of septic arthritis of the hips, osteomyelitis of the pelvic bones, pelvic abscesses, and septic thrombophlebitis[6]. Unlike hospital strains, which typically are resistant to multiple antimicrobial agents and share a common genotype with other hospital isolates, community acquired strains tend to be susceptible to non-beta-lactams and have genotypes distinct from hospital isolates in the same community[12]. Two main clones (USA 400 and USA 300, belonging to ST1 and ST8 by MLST, respectively) have been described as responsible for the majority of infections caused by CA-MRSA throughout the United States[12,25]. In other continents, the most frequent STs of CA-MRSA are the ST30 for the Southwest Pacific clone, and the ST80 for the European clone, although in Europe the ST8 and ST30 clones have also been described[13]. The *mecA* gene, the genetic determinant necessary for the expression of oxacillin resistance, resides in these strains on genetic elements (usually *SCCmec* types IV and V) that are different from those encoding methicillin resistance in hospital-acquired MRSA, although in recent years *SCCmec* type IV has been frequently found in nosocomial MRSA isolates mainly in Europe[9,26,27]. Another key difference is that almost all CA-MRSA isolates contain genes encoding the Panton-Valentine leukocidin, which is a cytotoxin that causes leukocyte destruction and tissue necrosis. Although the exact role of Panton-Valentine leukocidin in the serious infections caused by these organisms is unclear, it is in general a marker for CA-MRSA[13,28,29].

A hypothesis in order to explain the origins of CA-MRSA is that the *mecA* gene or the *SCCmec* have been transferred horizontally to one or more previously oxacillin-susceptible *S. aureus* strains that occupy traditional community niches. This possibility does account for the distinct phenotypic and genotypic characteristics of CA-MRSA. The relatively long time lag from the appearance of MRSA in hospitals to its emergence in the community may in part be due to the low frequency of horizontal chromosomal gene transfer[26]. At present, the knowledge of CA-MRSA epidemiology is incomplete which adds to the challenge of controlling the infection. In addition, nasal colonization may not precede CA-MRSA infections and since nasal carriage is not the prime predictor of subsequent infection it is more difficult to identify and control populations that are at risk. Colonization of the gastrointestinal tract and of household pets may act as additional reservoirs for CA-MRSA[30,31]. Moreover, CA-MRSA are now being introduced into hospitals, thus blurring the borders between community-acquired and hospital-acquired strains[25]. Therapy for infections due to CA-MRSA includes appropriate drainage of skin and soft-tissue lesions, since milder infections often respond to local incision and drainage alone[15]. The clinician should know when to use drugs with activity against CA-MRSA (clindamycin, minocycline, doxycycline, trimethoprim-sulfamethoxazole or vancomycin), although optimal antimicrobial agent therapy is unknown. For severe and invasive infections

therapy with vancomycin or linezolid should immediately start since antimicrobial agents may be ineffective when the patient receives treatment too late[6,15].

The recognition that partial vancomycin resistance may result in some vancomycin treatment failures for serious MRSA infections, portends the near-term loss of this first-line drug as the treatment of choice. *Vancomycin-intermediate S. aureus* (VISA) and heterogenous VISA (hVISA) have become a significant problem in many parts of the world[32-34]. VISA/hVISA isolates can arise from fully vancomycin-susceptible *S. aureus* during persistent infection that fails to respond to glycopeptide therapy and are associated with significant phenotypic changes[35]. Resistance may develop by different pathways, but these strains have a thickened cell wall with reduced peptidoglycan cross-linking leading to cell wall "clogging" with vancomycin. Moreover, a marked reduction in autolytic activity and reduced cell wall turnover have been found in VISA /hVISA strains[36]. Several studies have demonstrated a number of metabolic pathways and regulatory genes that may contribute to resistance[37]. In particular, the *agr* two-component regulatory system has been linked to low-level vancomycin resistance in *S. aureus*, with reports suggesting that *agr* type II strains and loss of *agr* function are associated with VISA[38]. It has also been noted that many reported VISA infections have involved biomedical devices, and biofilm formation on these devices could be an important initial step in the pathway to vancomycin resistance[39].

An "emerging" pathogen can be seen as a well-known pathogen for which newly discovered subpopulations of the parent strain are able to produce disease. *Staphylococcus aureus small-colony variants* (SCVs) fall into this category. This variant subpopulation is defective in electron transport, grow slowly, and produce colonies < 10% the size of the parent strain grown on the same medium for the same amount of time. Most of these clinical isolates cannot synthesize menadione or hemin, and these results in a block of electron transport at the level of menaquinone or the cytochromes[40]. SCVs might seem to be less virulent because of their slow growth and decreased production of coagulase and alpha-toxin. However, the intracellular location of SCVs shields them from host defenses, their slow growth reduces the efficacy of cell wall-active antibiotics, and a decreased membrane potential protects them from positively charged antimicrobials[41]. SCVs represent a subpopulation of *S. aureus* that can cause persistent and recurrent infections. While their overall prevalence has not been firmly established, problems in discovering and identifying these organisms may cause an underestimation or their prevalence[42].

Coagulase-negative staphylococci can also be seen as an "emerging" pathogen since it has acquired new virulence factors and resistance to new antimicrobials[43]. These microorganisms can colonize indwelling catheters (including central venous catheters) and form biofilms, that can result in bloodstream infections. The organisms embedded in a matrix of extracellular polymeric substances that they have produced exhibit an altered phenotype with respect to growth rate and gene transcription. Treatment of these infections with conventional antimicrobial agents alone is frequently unsuccessful due to the high tolerance of these agents in microorganisms comprising the biofilm[44]. Several preventative and treatment approaches have been investigated for catheter-related infections including a recent renewed interest in the use of bacteriophages for mitigating biofilm formation on indwelling catheters[45]. Recently, another cause of concern among coagulase-negative staphylococci is their resistance to new antimicrobial agents such as linezolid. Although resistance to linezolid is very infrequent among staphylococci, these organisms are able to acquire *de novo* resistance among patients who have a long duration of hospitalization and that have received high amounts of linezolid. Moreover, linezolid-resistant strains can be transmitted from patient-to-patient with their establishment as part of the skin flora and may be a precursor of linezolid-resistant MRSA[46].

Streptococcus pneumoniae is a cause of serious infections that are a major source of morbidity and mortality among all age groups in both the developed and the developing worlds. In addition, the emergence of strains with high-level resistance to penicillin and to other antimicrobial agents has raised concerns about the current and future effectiveness of antibiotic regimens. Nevetheless, there is now evidence of decreasing resistance to some antimicrobials in some regions of the world. Reduction in antimicrobial use in some community settings has been associated with a decline in resistance of pneumococci to some classes of antimicrobials, mainly beta-lactams. However, we are still witnessing a relentless increase in resistance to the macrolide class of antimicrobials[47]. Fortunately, control of pneumococcal disease through vaccination with the 23-valent polysaccharide vaccine in adults has demonstrated a reduction in risk of bacteremic pneumococcal disease after vaccination, as well as decreased risk of death and complications of hospitalization, which reinforces the need to improve compliance with existing pneumococcal vaccination recommendations for adults. Moreover, the introduction of the 7-valent pneumococcal conjugated vaccine not only prevents invasive disease in children but also appears to have resulted in a decline in the prevalence of resistant serotypes included in the vaccine, resulting in an overall decrease in the prevalence of pneumococcal resistance[48]. However, penicillin-nonsusceptible pneumococcal clones of nonvaccine serotypes have been reported as a cause of bacteremia and other infections. These clones may have been derived from capsular transformation of vaccine-related serotypes[49].

In recent years an upsurge in the incidence of *human listeriosis* seems to have occurred in some geographical areas. Although most cases are foodborne, the epidemiology is complex. Prevention of the disease must include dietary advice on avoiding high-risk foods routinely to pregnant women, to the elderly and to immunocompromised patients. Listeriosis manifests primarily as abortion, septicemia, or central nervous system infections with a high case-fatality rate in all patient groups. *Listeria monocytogenes* is the third most common cause of bacterial meningitis that occurs among immunocompromised patients and elderly individuals[50]. Symptoms and signs of patients presenting with *L. monocytogenes* meningitis are not different from those found in the general population of patients with community-acquired bacterial meningitis, albeit with a longer prodromal phase; however typical cerebrospinal fluid findings predictive for bacterial meningitis might be absent, and Gram stain has a low yield. In

patients aged > 50 years old or with risk factors for *L. monocytogenes* meningitis, an amoxicillin-based empirical antimicrobial regimen is mandatory, since this bacterium is resistant to cephalosporins.

Despite the availability, for decades, of meningococcal vaccines, *Neisseria meningitidis* remains a leading cause of meningitis, sepsis, and other serious infections in both industrialized nations and the developing world. Group C meningococcal conjugate–vaccine effectiveness in the United Kingdom declines from ~ 90% in the first year to 0% between 1 and 4 years after immunization in infants immunized at 2, 3, and 4 months of age and to 61% in toddlers given a single dose. A recent study[51] did not find evidence of lower immunity in children immunized as infants than as toddlers. On the basis of serum bactericidal activity and/or passive protection, 40–50% of both age groups are protected at 2-3 years after immunization, which is significantly greater than in unimmunized historical controls (< 5%). The study of Snape et al[52] on antibody responses to either a reduced dose of meningococcal C polysaccharide vaccine, which is meant to simulate exposure to *N. meningitidis*, or meningococcal C conjugate vaccine in healthy 13-15-year-olds in the United Kingdom who had been primed with a serogroup C conjugate vaccine 3-4 years previously, adds new data on the number of days required for antibody levels to increase after boosting with either of the 2 vaccines. In addition to susceptibility associated with flawed complement pathway function, several other pathways have also been convincingly linked to altered host susceptibility to meningococcal disease. The article by Jack et al[53] breaks new ground in increasing the understanding of the human genetic basis for some host defence failures in meningococcal disease. Surfactant protein (SP)–A and SP-D are pattern-recognition molecules of the respiratory tract that activate inflammatory and phagocytic defences after binding to microbial sugars. Variation in the genes of the surfactant proteins affects the expression and function of these molecules.

Routine use of *Haemophilus influenzae* type b (Hib) conjugate vaccines has dramatically decreased the incidence of invasive Hib disease in the western world. Despite the effectiveness of the vaccine, an increase in the incidence of Hib disease has recently been observed in some European countries. Careful analysis of circulating Hib strains is essential for prompt detection of any change in the properties of bacteria, enabling particular clones to overcome the host's immune response. In a recent Italian study[54], contrary to previous reports, neither increased genetic diversity of Hib strains isolated from children nor the disappearance of individual clones was observed after the rountine immunization of infants against Hib was established; however, an upward temporal trend in proportion of strains possessing multiple copies of the capsulation b locus was detected. The results of this study suggest that vaccine pressure may be positively selecting for strains that harbour amplified *cap b* sequences. Interactions of nontypeable *Haemophilus influenzae* (NTHI) with human alveolar macrophages are implicated in the persistence of NTHI in chronic obstructive pulmonary disease (COPD). A recent study by Sethi's group[55] confirmed the hypothesis that immunologic responses of alveolar macrophages to NTHI are impaired in COPD. The same group has described a phenotypic variant of NTHI, *Haemophilus haemolyticus* that frequently colonizes the airway of COPD patients and is not found in normally sterile sites. These findings substantially strengthen the association of true *H. influenzae* with clinical infection and exacerbation of chronic bronchitis[56]. The control of *pertussis infection* is not optimal, because neither vaccination nor natural infection induces long-lived immunity. Increasing proportions of reports of pertussis cases involve adolescents and adults. The aim of a recent US study was to define, among unimmunized control subjects, a yearly infection rate and to characterize the proportion of those infections that include prolonged coughs[57]. A similar analysis was attempted for acellular pertussis (aP)-vaccinated subjects. Pertussis infections in older persons was found to be largely asymptomatic; aP boosters confer protection for adolescents and adults against symptomatic pertussis.The diagnosis of pertussis in older individuals is problematic because of the lack of specific clinical criteria, insensitivity of culture and PCR, and the limited availability of standardized serologic tests and criteria for diagnosis. A Spanish study suggests that real time-PCR is the most sensitive and specific test available for the diagnosis of pertussis and that the direct fluorescent assay should be abandoned[58].

Three recent studies in *Escherichia coli* infections deserve comment. The first two deal with fluoroquinolone resistance in this species. The first shows the increasing prevalence of strains with reduced sensitivity to the quinolones in the gut of hospitalized patients[59], and the second analyses the differences and similarities of fluoroquinolone-resistant *E. coli* from humans and from chicken. The similarities among the strains studied strengthen the hypothesis of an animal origin of the human strains of fluoroquinolone-resistant *E. coli*[60]. A third study addressed the progressive importance of infections caused by ESBL-producing *E. coli*[61]. Bacteremia caused by these organisms is increasing and given the difficulties encountered in its treatment,-frequent multiresistance to other traditionally used agents,- a reappraisal of current empirical regimens is in order. A very good example of the difficulties found treating ESBL-producing organisms, is the case reported from Israel, a patient with an ESBL-producing *Klebsiella pneumoniae* endocarditis that developed resistance to ciprofloxacin and piperacillin/tazobactam while on therapy[62]. The presence of ESBL-producing *enterobacteriaceae* in the stools of humans, sewage and animals has been found to be high in areas where these studies have been done. From 20 to 100% of the farm animals examined in a recent Spanish study harbour ESBL-producing enteric bacteria[63]. The potential for dissemination of multi-resistance isolates is illustrated by two recent reports: the emergence of *Proteus mirabilis* carrying the bla metallo-beta-lactamase gene, the first instance in this species[64], and $bla_{\text{VIM-2}}$, and $bla_{\text{VIM-7}}$ carbapenemase-producing *Pseudomonas aeruginosa*[65]. Among the latter, the emergence of resistance to carbapenems can be very rapid; when associated with resistance to polimixins the problem at clinical level can become insoluble[66]. *Acinetobacter baumannii* has recently emerged as a major cause of hospital-acquired infection, because of its propensity to accumulate mechanisms of antimicrobial resistance that lead to pan-drug resistance and cause large nosocomial outbreaks that often involve multiple facilities. The problem is particularly serious in intensive care settings. An ex-

cellent review has been published recently on the epidemiology and control of *A. baumannii* infections in health care facilities[67]. Finally, although rare, community-acquired pneumonia due to *A. baumannii* is known to occur. A retrospective case-control study (cases: community-acquired pneumonia; controls: hospital acquired pneumonia) from Hong-Kong has been published[68].

Below, a group of Spanish physicians and microbiologists with an interest in the field of bacterial pathogens expand on the most remarkable papers published in this area during 2006. The following are the publications selected for discussion.

Community-acquired methicillin-resistant *Staphylococcus aureus*

Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al, for the EMERGEncy ID Net Study Group. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Eng J Med. 2006;355:666-74.

King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med. 2006;144:309-17.

Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF, King MD, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. Clin Infect Dis. 2006;647-56.

Klevens RM, Morrison MA, Fridkin SK, Reingold A, et al. Community-associated methicillin-resistant *Staphylococcus aureus* and healthcare risk factors. Emerg Infect Dis. 2006;12:1991-3.

These studies are an example of the CA-MRSA epidemic in the United States at present time. A population study conducted in three United States communities during 1997-1999 established the annual incidence of CA-MRSA to be 18-26 per 100.000, and most isolates were associated with clinically relevant infections. The current relevance of MRSA as a cause of skin and soft-tissue infections has been demonstrated in a prospective prevalence study involving patients presenting to emergency departments in 11 U.S. cities (Moran GJ et al. N Engl J Med. 2006;355). In this series, *S. aureus* was isolated in 320 of 422 pts (76%); 249 of them (78%) were MRSA. Overall, the prevalence of MRSA was 59%. The emergence of CA-MRSA USA 300 genotype as a predominant cause of skin and soft-tissue infections has been also shown in Atlanta (Georgia), an area of high prevalence of CA-MRSA (King M et al. Ann Intern Med 2006;144). In this active, prospective surveillance study, MRSA accounted for 72% of 389 episodes caused by *S. aureus*. Investigators from the same hospital in the Atlanta area reported the emergence of CA-MRSA USA 300 genotype as a major cause of health care-associated blood stream infections (BSI), in a prospective observational study (Seybold U et al. Clin Infect Dis. 2006;42). In this study, 132 episodes of BSI were documented over 7.5 months; notably, MRSA USA 300 caused both community onset and hospital onset BSI. An active, population-based surveillance for invasive MRSA infections involving 9 U.S. states, coordinated by the CDC, have shown that MRSA strains such as USA 300, which were initially a cause of MRSA infections in the community have migrated into health care settings (Klevens RM et al. Emerg Infect Dis. 2006;12). According to these observations, the distinction between healthcare and community-associated MRSA is rapidly blurring.

Comments

These studies demonstrate:

1) An increase in the incidence of skin and soft-tissue infections as well as in the incidence of invasive infections due to CA-MRSA.

2) The current predominance of the genotype USA 300.

3) The migration of CA-MRSA strains into the healthcare settings.

Hageman JC, Huyeki TM, Francis JS, et al. Severe community-acquired pneumonia due to *Staphylococcus aureus*, 2003-04 influenza season. Emerg Infect Dis. 2006;12:894-9.

Stapylococcus aureus is an infrequent cause of community-acquired pneumonia (CAP), but it is a recognized cause of influenza-associated CAP. MRSA commonly causes nosocomial pneumonia, but relatively few cases of MRSA CAP have been reported. During the 2003-2004 influenza season, 17 cases of CAP due to *S. aureus* were reported to the CDC from 9 states; 15 (88%) were caused by MRSA, and all isolates available for microbiological study presented genes for the Panton-Valentine leukocidine. Thirteen were admitted to the ICU, and death occurred in 5 patients. Authors suggest that empiric therapy of severe CAP during periods of high influenza activity should include coverage for MRSA.

Comments

In a country with high incidence of CA-MRSA, as it is in the United States, empiric therapy of severe CAP during periods of high influenza activity should include coverage for MRSA, even in patients without recognized risk factors for MRSA since they can be infected by a CA-MRSA strain.

Pannaraj PS, Hulten GH, Mason EO, Kaplan SL. Infective pyomiositis and myositis in children in the era of community-acquired, methicillin resistant *Staphylococcus* infection. Clin Infect Dis. 2006;43:953-60.

Pyomiositis is an acute bacterial infection of skeletal muscle with localized abscess formation caused in 75-90% of cases by *S. aureus*. Acute bacterial myositis is a less common muscle infection, but inflammation extends to more than one muscle group without distinct abscesses.

Although commonly caused by *Streptococcus pyogenes*, myositis caused by *S. aureus* have been described. Cases of pyomiositis and myositis have increased in pediatric patients since 2000 at Texas Children´s hospital, and this increase appears to correlate with the emergence of CA-MRSA. This study reviews the medical records of all patients admitted in this hospital from 2000 to 2005 with infective pyomyositis and myositis. Forty-five cases were analyzed. Forty percent had negative culture but 57.8% were caused by *S. aureus*. The number of cases increased from 2000 to 2005 as a result of an increase in the prevalence of CA-MRSA. The thigh and pelvis were the most commonly affected sites. Fifteen out of 24 available *S. aureus* isolates were CA-MRSA and 9 were community-acquired methicillin-susceptible *S. aureus* (CA-MSSA). By PFGE, 16 isolates were found to be USA300, and 17 carried the Panton-Valentine leukocidin (PVL) genes. Patients with CA-MRSA, USA300 and/or PVL-positive strains required more drainage procedures that did those with CA-MSSA, non-USA300 and/or PVL-negative strains (81 vs. 40%, 82 vs. 29%, and 81 vs. 38%, respectively). The authors strongly consider coverage for MRSA in the empirical treatment regimen (vancomycin or clindamycin) of these infections in children.

Comments

CA-MRSA is an increasing cause of pyomyositis and myositis in children. Although in this study the infections caused by CA-MRSA, USA300, and PVL-positive isolates were more severe than those caused by CA-MSSA, non-USA300, and PVL-negative strains, additional studies are needed to find out the virulence factors associated with muscle infection caused by CA-MRSA.

Zaoutis TE, Toltzis P, Chu J, Abrams T, Dul M, Kim J, et al. Clinical and molecular epidemiology of community-acquired methicillin-resistant *Staphylococcus aureus* infections among children with risk factors for health care-associated infection: 2001-2003. Pediatr Infect Dis J. 2006;25:343-8.

The epidemiology of CA-MRSA among healthy children has been recently described. However, little is known about CA-MRSA in children with underlying medical conditions. The authors compare in this study the clinical and molecular epidemiology of CA-MRSA in children with and without risk factors for health care-associated infections (RF-HAI). A 3-year retrospective cohort study of children with CA-MRSA infection was conducted. RF-HAI, including hospitalization within the past year, indwelling medical devices or chronic medical condition, were identified by chart review. The authors identified 446 episodes of community-acquired *S. aureus* infections, of which 134 (30%) were caused by MRSA. During the 3-year study period, the proportion of *S. aureus* infections caused by MRSA rose from 15% (12 of 80) to 40% (93 of 235) ($P < 0.001$) with the increase noted predominately in children with skin and soft tissue infections. RF-HAI were identified in 56 (42%) patients with CA-MRSA. Among subjects with CA-MRSA, children with RF-HAI were more likely to have had an invasive infection than healthy children (32 versus 5%; $P < 0.001$). CA-MRSA isolates from children with RF-HAI were similar to those without RF-HAI; all laboratory-retained CA-MRSA isolates harbored the SCC*mec* type IV cassette, and almost all isolates were susceptible to cotrimoxazole and clindamycin. PFGE revealed greater molecular diversity among CA-MRSA isolates recovered from children with RF-HAI compared with those from otherwise healthy children ($P = 0.001$). Additionally, CA-MRSA isolates from children with RF-HAI were less likely to be PVL-positive ($P < 0.001$) and more likely to be resistant to 3 or more classes of antibiotics ($P = 0.033$).

Comments

This study demonstrates that the borders between community-acquired and hospital-acquired MRSA strains are blurring, and suggests that CA-MRSA strains might have become endemic not only in adult health care facilities but also within paediatric facilities.

Coombs GW, Pearson JC, O' Brien FG, Murray RJ, Grubb WB, Christiansen KJ. Methicillin-resistant *Staphylococcus aureus* clones, Western Australia. Emerg Infect Dis. 2006;12:241-7.

CA-MRSA was first reported in Western Australia (WA) in the early 1990s from indigenous people living in remote areas. A statewide policy of screening all hospital patients and staff who have lived outside the state for MRSA has prevented the establishment of multidrug-resistant epidemic MRSA (mEMRSA), however, this study demonstrates that this policy has not prevented SCC*mec* type IV and type V MRSA clones (community clones) from becoming established in WA. All MRSA isolated in WA from July 2003 to December 2004 were included in this study. Isolates were recovered from clinical and infection control screenings. Of the 4,099 MRSA isolates analysed (those sent to a reference center), 77.5% were CA-MRSA. Using different molecular methods, a total of 22 different CA-MRSA clones were characterized. Of these isolates, 55.5% were resistant to ≥ 1 non-beta-lactam antimicrobial drug. Five PVL-positive CA-MRSA clones were identified.

Comments

The Australian policy that prevented the establishment of mEMRSA, has not prevented SCC*mec* type IV and V MRSA clones, including non-mEMRSA and CA-MRSA, from becoming established in WA. The emergence of multidrug-resistant CA-MRSA clones and the detection of PVL toxin genes in clones previously reported as PVL-negative is a major public health concern. Molecular typing is very important in tracing the origin of isolates and in designing antimicrobial drug prescribing policies for their control, particularly in the community.

Voyich JM, Otto M, Mathema B, Braughton KR, Whitmey AR, Welty D, et al. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? J Infect Dis. 2006;194:1761-70.

Some studies have indicated that CA-MRSA strains are generally more virulent than hospital-acquired MRSA, a

finding consisting with the ability of CA-MRSA to cause disease in individuals without predisposing risk factors. Although the molecular basis for the enhanced virulence is not known, there is strong association between CA-MRSA infections and the presence of PVL. However, the role that PVL plays in the pathogenesis of CA-MRSA has not been tested directly. In this study the authors evaluated, in a mouse infection model, the role of PVL in the virulence of CA-MRSA as well as the lytic activity and the intracellular survival in human polymorphonuclear leukocytes (PMNs) of isogenic strains of CA-MRSA, expressing or not PVL. They compared the virulence of PVL-positive with that of PVL-negative CA-MRSA representing the leading disease-causing strains. Unexpectedly, strains lacking PVL were as virulent in mouse sepsis and abscess models as those containing the leukotoxin. Isogenic PVL-negative (lukS/F-PV knockout) strains of USA300 and USA400 were as lethal as wild-type strains in a sepsis model, and they caused comparable skin disease. Moreover, lysis of PMNs and pathogen survival after phagocytosis were similar between wild-type and mutant strains.

Comments

Although the toxin may be a highly linked epidemiological marker for CA-MRSA strains, the authors conclude that PVL is not the major virulence determinant of CA-MRSA. It may be that PVL contributes to specific pathologic conditions such as necrotizing pneumonia, a disease not tested in this study. Probably future studies using other different animal models will help to understand the pathogenesis of CA-MRSA or to identify other factors responsible for the type and severity of disease caused by these emerging pathogens.

Staphylococcus aureus: epidemiology, resistance and virulence

LeBlanc L, Pepin J, Toulouse K, Ouellette MF, Coulombe MA, Corriveau MP, et al. Fluoroquinolones and risk for methicillin-resistant *Staphylococcus aureus*, Canada. Emerg Infect Dis. 2006;12:1398-405.

This paper presents a review of a cohort of 7421 patients admitted to four non-ICU hospital wards at a Canadian tertiary care centre from January 2003 to June 2004 in order to study the role of different antibiotics as risk factors for colonization and infection with MRSA. Patients with *S. aureus* infections present at or within 72 hours of hospital admission were excluded. Extensive socio-demographic data, discharge diagnoses and concurrent illnesses, drugs administered and laboratory results were recorded. Cox regression analysis yielded a significant association of nosocomial acquisition of MRSA colonization with older age, length of hospital-stay, peptic ulcer disease, and fluoroquinolone administration. Fluoroquinolones were the only antibiotic class being identified as independently associated with colonization and infection with MRSA (Hazard ratios 2.57, 1.84-3.60 and 2.49, 1.02-6.07, respectively). The authors suggest avoiding the use of fluoroquinolones and taking into account their prior use when instituting empiric antibiotic therapy.

Comments

The main limitation of this study is its retrospective nature, which cannot exclude bias. Unrecognized common factors leading to fluoroquinolone therapy and MRSA acquisition cannot be ruled out. In addition, adverse events associated with increased use of those antimicrobials substituting the fluoroquinolones need to be considered.

Charbonneau P, Parienti JJ, Thibon P, Ramakers M, Daubin C, du Cheyron D, et al. Fluoroquinolone use and methicillin-resistant *Staphylococcus aureus* isolation rates in hospitalized patients: a quasi experimental study. Clin Infect Dis. 2006;42:778-84.

The authors investigate the association between the use of fluoroquinolones and the recovery of MRSA in hospitalized patients. Four hospitals located in the same region of France with a similar pre-study incidence of MRSA are selected. The study manoeuvre consists of discontinuing the use of fluoroquinolones in one hospital for the study period, while the three others serve as controls without changing their antibiotic policies. As a consequence, the study hospital reduces the use of fluoroquinolones from 54 to 5 DDD per 1000 bed-days and increases the consumption of amoxicillin-clavulanate by 28%, ceftriaxone by 42%, gentamicin by 35%, amikacin by 25%, erythromycin by 65% and cotrimoxazole by 46%. During the intervention period the incidence of MRSA tended to be lower in the study hospital (Odds ratio 0.82; 95% CI 0.68-0.99). No reduction in the rate of fluoroquinolone resistance occurred in Gram-negative bacilli and the study centre suffered a nosocomial ESBL-producing *Klebsiella pneumoniae* outbreak during the intervention period.

Comments

The observed reduction in MRSA rate is significant, although small, in this interestingly designed study. Unfortunately, a spontaneous decrease of the incidence of MRSA, rather than due to the absence of the use of fluoroquinolones, may be responsible for the observed 18% reduction. In addition, a potential causal relation between the changes in antibiotic policy and the occurrence of an ESBL-producing *K. pneumoniae* outbreak is a matter of concern.

Howden BP, Johnson PD, Ward PB, Stinear TP, Davies JK. Isolates with low-level vancomycin resistance associated with persistent methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother. 2006;50:3039-47.

Low-level vancomycin-resistant *Staphylococcus aureus* (vancomycin-intermediate *S. aureus* –VISA– and heterogenous VISA –hVISA–) leads to glycopeptide treatment failure. The genetic changes leading to hVISA and VISA have not been clearly determined. This study characterizes five clinical pairs of fully vancomycin-susceptible *S. aureus* (VSSA) and hVISA/VISA (vancomycin MICs, 2 to 4 mg/L) isolates obtained before and after failed vancomycin therapy, in patients with bacteremia due to MRSA, in order to better understand the changes associated with this type of resistance. The hVISA/VISA phenotype was associated with increased cell wall thickness, re-

duced autolytic activity in four of five hVISA/VISA strains, and a striking reduction in biofilm formation compared to the parent strains in all pairs. All five pairs were isogenic, and genomic DNA microarray comparison suggested that major genetic changes are not required for the development of the resistant phenotype in these strains. A marked reduction in RNAIII expression of the agr gene was found in four pairs.

Comments

This study performs a detailed analysis of the different mechanisms that could explain the conversion of a VSSA into a VISA strain. Many of the phenotypic changes are consistent with previous reports; however, reduced autolytic activity is not essential for the expression of low-level vancomycin resistance in *S. aureus*. The use of pairs of clinically derived, isogenic strains of VSSA and hVISA/VISA will be a valuable resource to elucidate the genetic mechanism of low level glycopeptide resistance in the latter.

Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W. *Staphylococcus aureus* small colony variants in prosthetic joint infection. Cin Infect Dis. 2006;43:961-7.

Staphylococcus aureus small-colony variants (SCVs) are able to persist inside of host cells and to resist antibiotics. This characteristic is especially important when involved in implant-associated infections. The authors analyze 5 cases of hip-prosthesis-associated infections due to SCVs, including their course prior to identification of the pathogen. The patients' mean age was 62.2 years. All patients experienced treatment failures prior to isolation of SCVs, despite as many as 3 surgical revisions and up to 22 months of antibiotics. Transmission electron microscopy performed on biopsy specimens from periprosthetic tissue revealed intracellular cocci in fibroblasts. All prostheses were removed without implanting a spacer, antimicrobial agents were administered for 5.5-7 weeks, and reimplantation of the prostheses was performed for 4 patients. This 2-stage exchange was associated with successful outcome, with a mean follow-up of 24 months.

Comments

Slow growth, atypical colony morphology, and unusual biochemical profile make clinical laboratory personnel likely to miss or misidentify SCVs. In the case of a poor response to adequate antimicrobial and surgical treatment in implant-associated staphylococcal infections, the clinician and the clinical laboratory should consider special efforts to search for SCVs.

Cogulase-negative staphylococci: resistance and virulence

Potoski BA, Adams J, Clarke L, Shutt K, Linden PK, Baxter C, et al. Epidemiological profile of linezolid-resistant coagulase-negative staphylococci. Clin Infect Dis. 2006;43:165-71.

This study investigates an unusually high incidence of 4% linezolid resistance in coagulase-negative staphylococci (LR-CNS) at their centre in the USA. They perform an analysis of linezolid use and a retrospective case-control study to identify the risk factors and epidemiological profile of patients in whom LR-CNS had been identified. Each of the 25 case patients were first matched with 4 randomly selected concomitant patients at the same hospital ward. In a second analysis, each case patient was matched with 2 patients with linezolid-susceptible CNS isolated from clinical samples. The authors report that the use of linezolid had increased from 0.61 DDD per 100 bed-days in 2001 to 13.6 in 2005. In 19 of 25 (76%) patients with LR-CNS they detected prior use of linezolid. LR-CNS was identified in blood cultures of 5 patients, 14 times it was considered to be a contaminant and in 3 patients it was identified in 1 of 2 blood samples. All except one isolate (*Staphylococcus lugdunensis*) were identified as *Staphylococcus epidermidis*. MICs were higher than 256 mg/L and genotyping revealed relatedness of strains from 21 (84%) patients, 15 of whom had been admitted to the same ICU. Compared to random controls, prior treatment with linezolid was significantly associated with acquisition of LR-CNS (Odds ratio 20.6, 5.8-76), as was admission to ward "c" (Odds ratio 12.4, 3.4-45.5). Patients with linezolid-susceptible CNS had not been given linezolid (Odds ratio 0.09, 0.01-0.57).

Comments

This paper describes a clonal outbreak due to LR-CNS associated with extensive use of linezolid. Although the information provided is of interest, it is based on experience from only one centre, and it is a short series of 25 strains and only 5 clinical infections.

Curtin JJ, Donlan RM. Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis*. Antimicrob Agents Chemother. 2006;50:1268-75.

Comments

This *in vitro* study investigates the effect of a bacteriophage on biofilm formation by *Staphylococcus epidermidis* on hydrogel-coated Foley catheters. They demonstrate that pre-treatment of catheters with the lytic *S. epidermidis* bacteriophage 456 prevents formation of biofilm. While new technologies, such us pre-treatment of catheter surfaces with antiseptic or antibiotic agents show promise as prophylaxis/treatment of catheter-related infections, the results of this study highlight the potential of phages for the reduction of biofilm development on biomedical implant surfaces and suggest future developments in catheter-associated infection prevention.

Streptococcus pneumoniae: vaccines and resistance

Fisman DN, Abrutyn E, Spaude KA, Kim A, Kirchner C, Daley J. Prior pneumococcal vaccination is associated with reduced dead, complications, and length of stay among hospitalized adults with community-acquired pneumonia. Clin Infect Dis. 2006; 42:1093-101.

In spite of the antibiotic treatment and intensive care, the mortality of community-acquired pneumonia (CAP)

remains high. On the other hand, pneumococcal vaccination reduces the incidence of pneumococcal bacteremia, which itself has a high fatality rate. The objective of this study was to evaluate the impact of prior 23-valent pneumococcal vaccination (PV23) on the early mortality and complications in hospitalized adults with CAP. The study was carried out in 109 US teaching and community hospitals that share a common database.

A total of 62,918 individuals with CAP, identified by the INCD 9th rev. codes 480.0-487.0 entered the study during a five-year period (1999-2003). Data were obtained systematically using standardized definitions by trained nurses, concurrently with patient care, and validated monthly. Variables included those necessary for calculation of the Fine score, vaccination status (yes, 12%; no, 23%; unknown, 65%) and other comorbidities. No data on microbial etiology were recorded. Statistical analysis was done with multivariable logistic regression models.

Vaccine recipients were less likely to die during hospitalization that were unvaccinated patients (adjusted OR 0.50; 95% CI 0.43-0.59). This trend remained under varying assumptions about missing vaccination data. Vaccination also had a positive effect on the risk of respiratory failure (OR 0.67; 95% CI 0.59-0.76). Length of stay was also reduced in vaccinated patients ($P < 0.001$).

In conclusion, prior vaccination with the PV23 is associated with a 50% mortality reduction and a lower risk of complications, then reinforcing the efforts towards improving vaccination coverage in adults.

Comments

The absence of microbiological data, and the high proportion of individuals with unknown vaccination status were the main weaknesses of this observational study.

Withley C, Pilishvili T, Farley MM, Schaffner W, Craig AS, Lynfield R, et al. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched-control study. Lancet. 2006;368:1495-502.

This is a case-control study on the effectiveness of the 7-valent pneumococcal conjugate vaccine (PCV7) to prevent invasive pneumococcal disease in children 3-59 months old, including those vaccinated with incomplete schedules. The study was carried out in several urban areas and two entire states of the USA. Cases were identified by an active surveillance program operated by the CDC during 2001-2004. A total of 782 cases were included, and 3 controls per case, matched by age and zip code, were selected. A total of 485 cases were excluded, mostly those who had no isolate available for serotyping, or those whose parents refused to participate. Serotyping (Danish scheme), and susceptibility tests were done at CDC or at some state laboratories. Matched odds ratio (OR) was calculated by using conditional regression models controlled by underlying disorders, race, sex, and access to scheduled vaccinations, among other variables. Effectiveness was defined as one minus the adjusted matched ORx100%.

A total of 45% of cases were caused by serotypes included in the PCV7 (VT), mostly 14 and 19F, of which 26% were in children who had received al least one dose of the vaccine, and 8% were in fully vaccinated. Effectiveness in healthy children was 96% for VT strains, and 44% for vaccine-related serotypes [same serogroup but different type (VRT)]. Lower effectiveness was observed in children with underlying diseases (81% and 35% for VT and VRT, respectively. The level of protection was also lower for strains with decrease susceptibility to penicillin. The PCV7 did not prevent serotype 19A infection. Several schedules were more protective than no vaccination, being 3 doses plus a booster more protective than 3 doses alone. In summary, the PCV7 prevents invasive disease in both healthy and chronically ill children, even with various no standard schedules.

Comments

This study opens new doors to prospective studies for investigating simpler vaccination schedules. The vaccine fails with some serotypes and is less efficient with penicillin non-susceptible strains.

Steenhof AP, Shah SS, Ratner AJ, Patil SM, McGown KL. Emergence of vaccine-related pneumococcal serotypes as a cause of bacteremia. Clin Infect Dis. 2006;42:907-914.

In this observational study from the Children's Hospital of Philadelphia, the authors aimed to evaluate the effect of the PCV7 vaccine on the pneumococcal bacteremia (PnB) caused by VRT or by pneumococci non susceptible to penicillin (PNSP) in the post-licensure period of this vaccine. From January 1999 to May 2005, all episodes of PnB attended at the Pediatric Emergence Unit were revised retrospectively, as well as bacteremias caused by other respiratory bacterial pathogens (ORBP; *Haemophilus influenzae*, *Neisseria meningitidis*, and *Moraxella catarrhalis*), that were used as a non-equivalent dependent variable with the purpose of statistical analysis. Blood cultures were done according with the physician indication. Serotyping was performed with the Staten Seruminstitut antisera collection, and MICs to penicillin by using E-test strips (AB BioDisk). PCV7 vaccination status was no recorded among variables entering the study. An overall 57% decrease per year in the incidence of PnB was observed, mainly due to the reduction of cases caused by vaccine type strains, although a shift in this trend was noted in 2004-05. The incidence of PnB caused by non-vaccine types and ORBP remains stable, but that of VRT increased by 6% along the study period, being serotype 19A responsible for most cases. The percentage of PNSP strains also increased from 25 to 39% ($P < 0.05$).

Comments

This study calls attention on the possibility of serotype replacement after vaccination with the PCV7, and on the emergence of PnB caused by PNSP strains, possibly related with selection of some serotypes (19A) for which the PCV7 has poor immugenicity. In addition to its retrospective nature, the study has other limitations. The absence of records on the previous PCV7 vaccination in the study population, the use of a non-equivalent dependent variable for control of biases, and changing criteria in performing blood cultures along the study period (confound-

ing by indication) were the main weaknesses of the study, although the authors believe that some of these variables would be controlled in a certain extent. Therefore, prospective multicenter studies on this subject would be welcome.

Hsieh YC, Wang JT, Lee WS, Hsueh PR, Shao PL, Chang LY, et al. Serotype competence and penicillin resistance in *Streptococcus pneumoniae*. Emerg Infect Dis. 2006;12:1709-14.

After large-scale introduction of the PCV7 vaccine, two effects have become apparent. First, increasing pharyngeal colonization by serotypes not covered by the PCV7 (serotype replacement), and second, acquisition of a new nonvaccine capsule through recombination in naturally transformable clones (serotype swiching). Authors from Taiwan investigated the *in vitro* capacity of clinical strains of *S. pneumoniae* isolated in an area with low PCV7 coverage to become transformed (competence) *in vitro*. A total of 118 strains belonging to 7 serotypes were prospectively collected from Taiwanese hospitals from 2003 to 2005. With the exception of serotype 3, all strains were included in the PCV7. Competence studies were done with the use of two variants of the competence-stimulating peptide (CSP 1 and 2) and the pDL278 plasmid as a vector. Genetic diversity were also studied by pulse field gel electrophoresis (PFGE). Serotype 6B had the greatest competence, followed by types 14, 19F, 9V, 23F, 3, and 18C. Serotype 6B also showed the highest genetic diversity and higher proportion of penicillin nonsusceptibility rates. Conversely, strains belonging to types 3 and 18C showed the highest clustering, were mostly incompetent for transformation, and were 100% susceptible to penicillin. In spite of this qualitative association between serotype, competence and resistance, no correlation was observed between the level of competence and the degree of resistance to penicillin (OR 0.9; 95% CI 0.58-1.25). In this way, serotype 23F was relatively incompetent, but had a clear trend to higher proportion of resistant strains, and with higher penicillin MICs.

Comments

This study shows how certain serotypes with lower capacity to be transformed are genetically more stable, and why resistance to penicillin is rare in strains belonging to incompetent serotypes all over the world. Competence would be a necessary condition, but no sufficient, for explaining the genetic diversity and, secondarily the acquisition of penicillin resistance determinants in *S. pneumoniae*.

Metlay JP, Fishman NO, Joffe MM, Kallan MJ, Chittams JL, Edelstein PH. Macrolide resistance in adults with bacteriemic pneumococcal pneumonia. Emerg Infect Dis. 2006;12:1223-30.

Streptococcus pneumoniae is the leading cause of CAP in adults. Bacteremic pneumococcal pneumonia (BPP) is among the most serious forms of pneumococcal disease. This population-based case-control study identifies clinical and demographic factors associated with macrolide-resistant BPP in adults from 43 acute-care hospitals at the Pennsylvania region. From December 1, 2000, to April 17, 2004 patients included in the study were individuals who 1) were 18 years or more; 2) had at least one blood culture that grew *S pneumoniae* drawn within 48 hours of hospital admission; 3) resided in one of the five counties surrounding Pennsylvania, and 4) had a bacterial isolate confirmed in the laboratory as *S pneumoniae*. Seventy-six patients had erythromycin-resistant infections and were selected as the case-patients for this study. The authors found that exposure to macrolides in the six months preceding infection, a history of influenza vaccination in the previous year, and Hispanic ethnicity were all independently associated with an increased probability of erythromycin –resistant *S. pneumoniae* infection. Among patients who reported taking antimicrobial agents in the 6 months preceding infection, failure to complete the course of prescribed drugs was associated with an increased probability of macrolide resistance ([OR = 3.4]; 95% CI 1.2-9.9). However, most patients with macrolide-resistant infections did not report any prior antimicrobial drug exposures.

Comments

The authors assume in the discussion potential biases that may have affected the assessment of different risk factors. Future studies correlating duration of therapy with risk for colonization with macrolide-resistant pneumococci would be useful to further explore this phenomenon.

Streptococcus pneumoniae: coinfection with viruses

Watson M, Gilmour R, Menzies R, Ferson M, McIntyre P, for the New South Wales Pneumococcal Network. The association of respiratory viruses, temperature, and other climatic parameters with the incidence of invasive pneumococcal disease in Sydney, Australia. Clin Infect Dis. 2006;42:211-5.

The incidence of invasive pneumococcal disease (IPD) in children and adults increases in winter, however possible underlying causes have not been carefully studied. This ecological study correlated population-based data on IPD and respiratory virus activity in the year 2000 in metropolitan New South Wales, Australia, with climatic parameters. Seven hospitals participated in the study from May to October. During a year with good separation of respiratory syncytial virus (RSV) and influenza virus activity, it was demonstrated in children a significant correlation with RSV activity and IPD activity, but no significant correlation between influenza virus activity and IPD. In adults the epidemic curves of RSV and influenza virus activity corresponded with peaks of IPD, but it was only possible to demonstrate a statistically significant correlation when the activity of both viruses was combined. Of the climatic parameters there was a clear inverse relationship between weekly mean minimum and maximum temperatures and IPD activity in adults and children.

Comments

Data from other temperature geographic areas and data obtained after the introduction of vaccines are required to confirm these findings. Probably a case-control study comparing the isolation of respiratory viruses in patients with IPD matched with age, sex, location and time with control subjects without IPD would be required to specifically examine the role that respiratory viruses play in predisposing to IPD. The effect of falling temperatures on the pathogenicity of *S penumoniae* has not been extensively studied and warrants further investigation.

Madhi SA, Ludewick HL, Kuwanda L, Van Niekerk N, Cutland C, Little T, et al. Pneumococcal coinfection with human metapneumovirus. J Infect Dis. 2006;193:1236-43.

Human metapneumovirus (hMPV) has been found to be associated with lower respiratory tract infections although the pathogenesis remains to be elucidated. There are few reports of respiratory tract infections due to bacterial coinfection with hMPV. This hypothesis-generating study, performed from March 1998 to October 2002, involved a cohort of 39,836 children in South Africa randomized to receive the 9-valent pneumococcal polysaccharide-protein conjugate vaccine (PCV9) or placebo. These that were hospitalised (3.069) for lower respiratory tract infection (LRTI) were tested for hMPV infection. By use of a nested reverse-transcription PCR assay targeted at amplifying a fragment of the hMPV fusion protein gene, 202 such infections were identified among 2715 episodes of LRTI in children. In fully vaccinated children, the incidence of hospitalization for a least one episode of hMPV-associated LRTI was reduced by 46% ($P = 0.0002$) overall, by 45% ($P = 0.002$) in human immunodeficiency virus (HIV)- uninfected children, and by 53% ($P = 0.035$) in HIV-infected children. There was a significant reduction in the incidence of clinical pneumonia among vaccine recipients overall (58%; $P = 0.0001$), in HIV-uninfected children (55%; $P = 0.003$), and in HIV-infected children (65%; $P = 0.02$). There was no significant reduction in the incidence of hospitalization for hMPV-associated bronchiolitis among vaccine recipients in the entire study population.

Comments

The absence of sensitive tools to diagnose bacterial pneumonia has been a major obstacle in order to define the role of bacterial-virus coinfection in humans. It has been documented that approximately one-third of children with respiratory syncytial virus associated pneumonia may have pneumococcal coinfections. The results of the present study suggest that bacterial coinfections, particularly pneumococcal infections, are an essential part of the pathogenesis of most severe hMPV infections progressing to pneumonia. An implication of this observation is that children hospitalized with the diagnosis of hMPV-associated pneumonia should be treated with antibiotics, and a significant proportion of these hospitalisations may be prevented by vaccination with pneumococcal vaccine. Nevertheless more information is needed in order to clarify the pathogenic mechanisms of the mixed infections.

Listeria monocytogenes

Brouwer MC, van de Beek D, Heckenberg SGB, Spanjaard L, de Gans J. Community-acquired *Listeria monocytogenes* meningitis in adults. Clin Infect Dis. 2006;43:1233-8.

In this study, the authors describe the clinical features, complications, treatment and outcome of 30 episodes of community-acquired *Listeria monocytogenes* meningitis in adults. These cases were included in a prospective nationwide observational cohort study of bacterial meningitis with a positive LCR culture, performed in the Netherlands between October 1998 and April 2002. Over the period of study, 696 cases of bacterial meningitis were diagnosed and these caused by *L. monocytogenes* represented 4%. The annual incidence *L. monocytogenes* meningitis was 0.07 cases per 100,000 adults. All patients were immunocompromised (67%) or > 50 years old. In 19 (63%) of 30 patients, symptoms were present for > 24 h, and the clinical presentation was subacute in 8 patients (27% had symptoms for ≥ 4 days). The classical triad of stiff neck, fever and alterations in mental status was present in 43% of the cases. Gram staining of cerebrospinal fluid (CSF) samples revealed the causative organism in 7 (28%) of 25 cases and a biochemical CSF indicator of bacterial meningitis was present in 23 (77%). The initial antimicrobial therapy was amoxicillin based for 21 (70%) of 30 patients. The coverage of initial antimicrobial therapy was microbiologically inadequate for 9 (30%) of the patients. The mortality rate was 17% (5 patients), and 8 (27%) experienced an unfavourable outcome. Inadequate initial antimicrobial therapy was not related to outcome.

Comments

The study have limitations due to the selection of patients with a positive *L. monocytogenes* CSF culture. The most severe patients (in which antimicrobial treatment is started before the CSF is obtained or these with important neurological alterations) might have been excluded. Nevertheless, this prospective study confirms that typical CSF findings predictive for bacterial meningitis might be absent (biochemistry and Gram stain), but in contrast with previous reports, patients with meningitis due to *L. monocytogenes* do not present with atypical clinical features. It is interesting to note the high rate of patients (83%) with hyponatremia and the development of a subarachnoidal hemorrhage in one patient, complications more frequently related with tuberculous meningitis.

Gillespie IA, McLauchlin J, Grant KA, Little CL, Mithani V, Penman C, et al. Changing pattern of human listeriosis, England and Wales, 2001-2004. Emerg Infect Dis. 2006;12:1361-6.

The authors review the microbiologic and epidemiologic data of 1,933 cases of human listeriosis reported in England and Wales from 1990 to 2004. Ten common-source outbreaks affecting 60 patients were excluded from the study. A significant increase was observed during the period 2001-2004 compared to 1990-2000. The majority of the cases were sporadic (1,873) and not pregnancy-related (1,155). Deaths were more frequent in the group of non-pregnancy-related cases (44 vs. 10%). The majority of the

sporadic cases diagnosed between 2001 and 2004 occurred in patients > 60 years of age with bacteriemia without CNS affectation and without relation to sex, race, season of the year, socioeconomic differences or underlying conditions. Serotypes 4b and 1/2a were the most frequent throughout the entire study.

Comments

The increase of cases of listeriosis shown in the second period analysed in this study could be due to an increased interest in reporting this illness. Nonetheless, a change in the pathogenicity of *L. monocytogenes* could also explain the results. The advice to avoid high-risk foods for people of advanced age and for those that are immunocompromised, and not only for pregnant women, is a plausible message of this study.

Neisseria meningitidis

Vu DM, Kelly D, Heath PT, McCarthy ND, et al. Effectiveness analysis may underestimate protection of infants after Group C meningococcal immunization. J Infect Dis. 2006;194:231-7.

The immunized toddlers have a higher meningococcal antibody in serum samples than immunized infants. The authors determined meningococcal antibodies in serum samples obtained from children who were immunized with Group C meningococcal vaccines 2-3 year earlier as infants or toddlers.The geometric mean serum antibody concentrations were higher in immunized infants or immunized toddlers than in un-immunized historic control (0.82 and 0.56 mcg/ml vs. 0.08 mcg/ml, respectively; $P <$ .0001). The proportion of immunized infants who had bactericidal titers ≥ 1:4 (considered to be protective when measured with human complement was higher than of immunized toddlers (61 vs 24%; $P < .01$). Even if threshold for protection is considered to be a serum bactericidal titer ≥ 1:8 then the respective percentages of serum samples with protective titers is 50% for immunized infants, compared with 16% for the immunized toddlers ($P = .0006$). When passive protective was tested, 50% of serum samples from immunized infants and 41% of serum samples from immunized toddlers conferred protection against group C bacteremia, compared with 3% of serum samples from unimmunized historic controls ($P < .0001$).

Comments

There was no evidence of lower immunity in children immunized as infants than as toddlers. On the basis of serum bactericidal activity and/or passive protection, 40-50% of both age groups are protected at 2-3 years after immunization, which is significantly greater than in unimmunised historical controls (< 5%).

Snape MD, Kelly DF, Salt P, Green S, et al. Serogroup C meningococcal glycoconjugate vaccine in adolescents: persistence of bactericidal antibodies and kinetics of the immune response to a booster vaccine more than 3 years after immunization. Clin Infect Dis. 2006;43:1387-94.

The aims of this study were to determine hSBA (human serum bactericidal assays) GMTs (geometric mean titers) and the percentage of participants with an hSBA titer ≥ 8 at 2-7 days after vaccination with MenPs. Secondary endpoits were to determine the hSBA GMT and geometric mean antibody concentration measured by ELISA for the following populations: 1) all vaccine recipients (at day 0); 2) all recipients of MenPS and MenCV (analyzed separately for values at both day 0 and day 28 after vaccinations), and groups 1-6 (analyzed separately for all days on which blood samples were obtained)

This was a phase IV, open-label randomized comparative trail. Healthy 13-15 year olds who were vaccinated with a single dose of Menjugate (Chiron Vaccines), a meningococcal serogroup C-CRM_{197} glycoconjugate vaccine (MenCV) in the 1999-2000. Previous immunization status was determined by reference to the centralized immunization records of the relevant Child Health Computer departments. Subject numbers were prospectively randomized in blocks of 6 according to computer-generated blocked randomization scheme that allocated equal numbers of subjects to the 6 groups. Participants randomized to groups 1, 2, 3, or 4 received the plain polysaccharide serogroup A and C meningococcal vaccine (MenPS), where persons randomized to groups 5 or 6 received MenCV. A one-fifth dose of MenPS was used. Blood samples of up to 10 ml were obtained prior vaccination, twice more in the week after vaccination and at day 26-28 after vaccination. Serum samples were analyzed for MenC SBA titer using hSBA. An hSBA titer ≥ 8 was used to indicate a very conservative measure of protection.

A total of 274 participants were randomized to 1 of 6 groups. One hundred seventy-one of these had been randomized to group 1-4 (received MenPS) and 89 randomized to groups 5 ot 6 (received MenCV). An hSBA titer ≥ 8 was measured in the serum samples of 74.6% of participants. No increase in hSBA GMT was detected until 5 days after administration of MenPS or MenCV. All participants demonstrated an hSBA titer ≥ 8 at day 28 after vaccination. The hSBA GMTs observed 28 days following vaccination with MenCV (4979.4) were higher than those observed following vaccination with MenPS (2370.9). ELISA GMCs were similarly higher 28 days after vaccination with Men C or MenPS (35.7 vs. 19.5, respectively).

Comments

This study shows persistence of sustained levels of bactericidal antibodies for at least 3 years after vaccination of adolescent with MenCV. After challenge of immunized adolescents with MenPS, there was no increase in SBA observed until days 5 after vaccination, indicating that immunological memory may be too slow to generate protection against this potentially rapidly invasive organism.

Jack DL, Cole J, Naylor SG, et al. Genetic polymorphism of the binding domain of surfactant protein-A2 increases susceptibility to meningococcal disease. Clin Infect Dis. 2006:43:1426-33.

The distribution of polymorphisms in three genes (SP-A1; SP-A2 and SP-D) in a cohort of patients with microbiologically proven meningococcal disease was studied.

This study was realized in a cohort of patients with proven meningococcal disease. From July 1998 through

November 1999, all whole-blood samples obtained from patients were stored. The serogroup of the infecting organism, the disease outcome and the age of patients were recorded. The control group comprised healthy volunteers. Samples were chosen at random from the cohort for SP-A/SP-D genotyping. In total, 302 patients were genotyped for polymorphisms at all loci in SP-A2, and 303 for SP-A1. Data for SP-D polymorphisms were available for 294 patients. In the control group, 218 and 222 individuals were typed successfully for SP-A2 and SP-A1 respectively, and 227 were typed for SP-D. Two alleles of SP-A2 had a significant association with susceptibility to meningococcal disease. Homozygosity for 1A was associated with an increased risk of meningococcal disease (OR 7.4; 95% CI 1.3-42.4), with a 6.3% of the patient population homocygous for 1A compared with only 0.9% of the control population. Homozygosity for the rare SNP (SP-A2 at codon 223) was associated with an increased risk of meningococcal disease (OR 6.7; 95% CI 1.4-31.5) and increased risk of death (OR corrected for age 2.9; 95% CI 1.1-7.7). Haplotype 6A/1A was higher in patients (4.1%) than in control subjects (1.5%; OR 2.35 95% CI 1.28-4.31).

Comments

Gene polymorphism of the binding domain of surfactant protein A-2, SP-A2 at codon 223, increases susceptibility to meningococcal disease, as well as the risk of death.

Haemophilus influenzae

Cerquetti M, et al. Genetic diversity of invasive strains of *Haemophilus influenzae* type b before and after introduction of the conjugate vaccine in Italy. Clin Infect Dis. 2006;43:317-9.

In the era of universal vaccination of children with the *H. influenzae* conjugate vaccine, it is critical to characterize the currently circulating strains for detection of any genetic change that could render the current vaccine no longer protective. In Italy, this vaccine is included in the vaccine calendar since 1999, ant the uptake of the population exposed has been > 50% since 2000.

The aims of this study were to evaluate the genetic and capsular structures of the available strains from patients with invasive disease from 1997 to 2003, to assess the influence of vaccination in the changes observed in these structures..

The genetic diversity of the 95 strains was analysed by PFGE and capsular changes by Southern blot. The 60 strains isolated pre licensure (1997-1998) was compared with the 35 strains isolated post licensure (1999-2003).

The 95 strains had by PFGE a total of 28 restriction patterns, with a clear predominante of 5 of them. It should be stressed that almost half of the strains had a restriction pattern indistinguishable from 40F, the dominant and endemic clone in Italy since 1994. Four strains were fromchildren that had received at least one dose of the vaccine. A high genetic homology was detected in 90.5% of the isolates. The amplification of the locus of capsulation b showed that there is a significant difference between the two analysed periods. Thus, the strains from the vaccine period, including those from children previously vaccinated, had a number of copies higher than 2 in 54,3% of the cases, compared to 33,3% in the prevaccine period (P = 0,046).

Comments

The results of this study indicate the importance of monitoring invasive strains. The use of molecular genetics applied to this end, has shown that vaccination has not conditioned the occurrence of genetic changes nor the disappearance of dominant clones; it has been followed, however, by relevant changes in the expression of the capsulation genes, that could explain, at least in part, the lack of immune response in some vaccinated children.

Murphy TF, et al. *Haemophilus haemolyticus*: A human respiratory tract comensal to be distinguished from *Haemophilus influenzae*. J Infect Dis. 2007; 195:81-9.

Non-typable *Haemophilus influenzae* (NTHI) is a frequent pathogen in patients with COPD. When acute exacerbations are due to bacterial infection, NTHI are the most frequent isolated organisms.

In this report, 490 NTHI isolates from a variety of respiratory sources, were studied. Also, the microbiological results of 118 COPD patients of monthly sputa collected prospectively or in case of exacerbation, collected during a period of 9 years, were evaluated. Identification and typing were done by standard methods. Phenotypic variants were identified as *H. haemolyticus* with 4 independent methods: análisis of rDNA sequence, MLST, hybridization DNA-DNA y sequencing the gene encoding protein P6.

Of a total of 490 strains studied, 39.5% of sputum isolates and 27.3 of nasopharingeal isolates had genotypic characteristics of *H. haemolyticus*. None of the invasive isolates studied had the genotypic characteristics of *H. haemolyticus*. Molecular typing of sputum isolates made possible to demonstrate a statistically significant difference in the acquisition of a new strain of *H. influenzae* as a causal agent of an exacerbation in patients with COPD (44.5 vs 16.5%; $P < 0.0001$; RR 4.09 IC 95% 2.89-5.80). The differences in the case of *H. haemolyticus* were not significant (20.7 vs. 17.4%; $P = 0.41$; RR 1.24 IC 95% 0.74-2.07).

Comments

From the results of this study, it can be inferred that *H. haemolyticus* is a frequent inhabitant of the respiratory tract of children and adults with COPD, as a commensal, without pathogenic capacity. Unfortunately, with the standard methodology used for identification of *H. influenzae* it is not possible the identification of the phenotypic variant *H. haemolyticus*, a fact that might have important clinical implications in the use of antibiotic treatment in many patients.

Berenson CS, et al. Impaired phagocytosis of nontypeable *Haemophilus influenzae* by human alveolar macrophages in chronic obstructive pulmonary disease. J Infect Dis. 2006;194:1375-84.

The persistance of non-typable *Haemophilus influenzae* (NTHI) in patients with COPD is thought to be related with interactions between this bacterium and human alveolar macrophages. The immune mechanisms that mediate this macrophage response are poorly known.

The aims of this study were to demonstrate the importance of the alteration of the immune response of alveolar macrophages in the persistence of NTHI in the lower airways in patients with COPD. Three groups of patients were included: esmokers with COPD (n = 14), esmokers without COPD (n = 15) and non-smokers (n = 9). Respiratory and blood samples were used for separation of purified macrophages. Phagocytosis of adherent cells and survival of intracellular NTHI, three different strains of NTHI from patients with COPD were used.

Alveolar macrophages of COPD patients had diminished phagocytosis as compared with with macrophages from the other two groups in front of the three strains. However, phagocytosis of NTHI by blood macrophages was similar in all three groups studied. Finally, intracellular survival of NTHI was not altered in the alveolar macrophages of the patients with COPD.

Comments

The results of the study demonstrate the existence of an alteration in the immune response of alveolar macrophages of patients with COPD vs. NTHI, as sown by a diminution of phagocytosis, that explains the persistence of this organism in the airways. The absence of alterations in blood macrophages indicates the existence of a compartmentalised immune response in this population. However, intracellular lysis of NTHI is not altered in alveolar macrophages of COPD patients

Bordetella pertussis

Ward et al. *Bordetella pertussis* infections in vaccinated and unvaccinated adolescents and adults, as assesed in a national prospective randomized acellular pertussis vaccine trial (APERT). Clin Infect Dis. 2006;43: 151-7.

The control of pertussis infection is not optimal, because neither vaccination nor natural infection induces long-lived immunity. The estimated annual incidence of clinical pertussis infection in USA in adolescents and adults is about 370-500 cases per 100,000 person-years. The lack of specific clinical criteria and the limited availability of standardized serologic criteria make difficult the diagnosis of pertussis infection. A study to determine the impact of vaccination in adolescents and adults, as a part of a National Institutes of Health-sponsored multicenter, prospective, randomized acellular pertussis vaccine (aP) efficacy trial in the US, was recently reported. USA.

The study was performed in 2781 adults (15-65 years-old) in which one-half of the subjects received aP vaccine and one-half received hepatitis A vaccine (control subjects). All patients were observed for 2 years for clinical illness (telephone calls every 14 days) and serological studies obtained at baseline, +1 month and +12 months after vaccine application. For serological evaluation IgA and IgG antibodies to pertusis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN) (antigens all included in vaccine), and fimbriae 2/3 (FIM) (not included in vaccine), were quantitated by ELISA.

Seroconversion rates for each antigen (PT was the most specific) ranged from 0.4% to 2.7% in unvaccinated patients. Authors estimate that the infection rate among unvaccinated adults is close to 1% over an 11-month period. In vaccinated patients the infection rate and the efficacy of vaccine are difficult to obtain. Only 0.47% of vaccinated patients had titers at 1 year that were equal to or higher than the titres 1 month after immunization and only 0.23% of patients a 3-fold cut-off. FIM antibodies (antigen not included in vaccine) were significantly less common among vaccinated subjects than among controls ($P < 0.046$) suggesting that infection was less frequent (although marginally significant) in vaccinated patients. In vaccinated and unvaccinated patients symptoms probably related to pertussis infection were more common in patients who had serologic evidence of infection.

Comments

The incidence of *B. pertussis* infection in adults is about 1% per year and is usually asymptomatic. *B. pertussis* boosters may confer protection for adolescents and adults against symptomatic pertussis infection and may reduce transmission to others.

García-Martínez J, et al. *Bordetella pertussis* detection by real-time PCR, immunofluorescence and culture: prospective evaluation and molecular epidemiology. Enferm Infecc Microbiol Clin. 2006;8: 500-4.

The diagnosis of pertussis infection is problematic because of the lack of specific clinical criteria, insensitivity of culture and PCR and the limited availability of standardized serology tests.

The aim of this study was to determine the usefulness of several procedures, including real-time PCR, for the laboratory diagnosis of pertussis, and to investigate clonal relationships among clinical isolates of *Bordetella pertussis*.

The study was performed in one tertiary hospital in Madrid, Spain. During 15 months (August 2002 to October 2003) nasopharyngeal swabs were collected from paediatric and adult patients with symptoms of pertussis, and contact cases. The samples were processed by culture (Regan-Lowe medium), direct fluorescence assay (with polyclonal antibodies against wall antigens), and real-time PCR (light-Cycler, primers BP1, region IS481). Most of the isolates were further characterized by pulsed-field gel electrophoresis.

Among 121 clinical samples corresponding to 117 patients, *B. pertussis* was detected in 17 samples by culture (14.1%), 30 samples (24.8%) by DFA and 41 samples (33.9%) by real-time PCR. Real-time PCR diagnosed 26 and 24 more cases than culture and DFA, respectively. In relation to culture, sensitivity and specificity of PCR were 88.2% and 75%, respectively. Authors consider that a probable partial immunity in patients may justify better results for PCR in relation to culture. Seventeen clinical isolates were available for PFGE analysis and 5 different genotypes were identified. Fourteen isolates were included in two genotypes (genotype C and genotype E).

Comments

Real-time PCR applied to the diagnosis of pertussis provides more positive results than DFA and culture. On the basis of these results, DFA should no longer be used for the diagnosis of *B. pertussis* infection. A 2 to 5 years cycle is observed in relation to *B. pertussis* infection, independently of vaccine programmes. Some clones are dominant and one of them has circulated at least since 1997.

Enterobacteriaceae

Lautenbach et al. Phenotypic and genotypic characterization of fecal *Escherichia coli* isolates with decreased susceptibility to fluoroquinolones: results from a large hospital-based surveillance study. J Infect Dis. 2006;194:79-85.

The aims of this study were to determine the prevalence of clinical isolates of *E. coli* resistant to the fluoroquinolones in hospitalizad patients, as well as the underlying mechanisms of resistance (mutations in genes *gyrA* and *parC*), and tolerance to organic solvents as markers of of overexpression of the active efflux system acrAB. E. coli with a levofloxacin MIC > 0,125 mg/L were studied. Of the 789 fecal samples analysed, 149 (18.9%) *E. coli* with diminished fluoroquinolone susceptibility were isolated (18.9%). Of these 149 isolates, 102 had a levofloxacin MIC > 8 mg/L, these strains had a mean of 3 mutations in genes *gyrA* and *parC* and presented tolerance to organic solvents more frequently than the isolates with a levofloxacin MIC < 8 mg/L that exhibited a mean of one mutation in *gyrA*. The prevalence of isolates with tolerance to organic solvents varied during the period of study. No clonal dissemination was detected.

Comments

The main conclusions of this study are that colonization of the gut by *Escherichia coli* with reduced susceptibility to quinolones in hospitalizad patients is common, and that the resistant determinants to the quinolones vary over time. Resistance to nalidixic acid can be used in the identification of strains of *E. coli* with mutations in *gyrA*.

Johnson et al. Similarity between human and chicken *Escherichia coli* isolates in relation to ciprofloxacin resistance status. J Infect Dis. 2006;194:71-8.

Comparative study of the molecular epidemiology of *Escherichia coli* of humans (35 blood isolates and 33 faecal isolates) and chicken (49 faecal isolates) that were susceptible (n = 57) o resistant (n = 60) to ciprofloxacin, where an analysis of the filogenetic group, virulence genotype and the epidemiologic relationship with RAPD and PFGE were carried out. Among human isolates, those resistant to ciprofloxacin were different of the ciprofloxacin-resistant strains, while the sensitive and resistant isolates of chicken were indistinguishable. Susceptible human isolates had more genes associated with virulence factors than resistant human isolates or susceptible or resistant chicken isolates. Some resistant human isolates were very similar to some chicken isolates by RAPD and PFGE.

Comments

This is another study that increases the degree of evidence to the hypothesis that ciprofloxacin resistant isolates can appear *de novo* (after selection pressure by enrofloxacin and other quinolones used in animal husbandry), in susceptible progenitors in the animal's gut, then be transmitted to humans via the food chain and, finally cause infections in humans with ciprofloxacin-resistant strains.

Rodríguez-Baño J, Navarro MD, Romero L, et al. Bacteremia due to extended-spectrum β-lactamase-producing *Escherichia coli* in the CTX-M era: a new clinical challenge. Clin Infect Dis. 2006;43:1407-14.

This is a retrospective study of the predisposing factors, clinical presentation and evolution of 43 episodes of ESBL-producing *Escherichia coli* bacteraemia seen from January 2001 to March 2005 in a single institution; it represents a 8,8% of the total cases of *E. coli* bacteraemia seen during the same period. 70% of ESBL-harbouring *E. coli* produced CTX-M types and the great majority was not clonally related; 49% were of nosocomial origin, 32% were from geriatric centers, and 19% from the community. The number of cases increased from 6 in 2001 to 16 in 2004. The mean age of the infected patients was 71, 70% were male, 33% had a Foley catheter, 40% had urinary tract obstruction and 31 patients (72%) had received antimicrobials in the recent past (aminopenicillins, third generation cephalosporins, and fluoroquinolones).Crude mortality was 21%. Patients treated with a combination of β-lactam/β-lactamase inhibitor or a carbapenem had a mortality of 9% as compared with a mortality rate of 35% in those treated with cephalosporins or fluoroquinolones (P = 0.05). The failure rate of those treated with a cephalosporin was independent of the MIC values; however the number of patients studied was small and this finding has o be interpreted with caution.

Comments

In areas where the prevalence of ESBL-producing *E. coli* is increasingly represented, therapeutic guidelines should be revised. Given the characteristics of the population described, this suggerence is particularly important when sepsis is diagnosed in old males, carriers of a urinary catheter and those that had been treated with antimicrobials in the recent past.

Zimhony O, Chmelnitsky I, Bardenstein R, et al. Endocarditis caused by extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*: emergence of resistance to ciprofloxacin and piperacillin-tazobactam during treatment despite initial susceptibility. Antimicrob Agents Chemother. 2005;50: 3179-82.

The auhors decribe the case of a 45 years old woman with a prosthetic mitral valve that had a *Klebsiella pneumoniae* bacteraemia secondary to an infected intravenous catheter. The initial isolate produced An ESBL, susceptible to ciprofloxacin (MIC, 0.38 mg/L) and piperacillin/tazobactam (PT). Patient was with a combination of ciprofloxacin

and amikacin, and had recurrent bacteraemia 15 days later. This time the blood isolate was resistant to ciprofloxacin (MIC, 6 mg/L) and an echocardiogram showed vegetations in the prosthetic valve. Ciprofloxacin was replaced by piperacillin/tazobactam. However, after two weeks treatment bacteraemia persisted and repeat blood cultures grew PT resistant *E. coli*. Finally, the patient underwent prosthetic valve replacement and was treated with meropenem.

The three isolates were genotypically identical. The resistance to ciprofloxacin was associated to a mutation in *gyrA*. The activity of PT against inolcula of 10^5 and 10^7 UFC/ml showed that with low inocula, the first two isolates were sensitive to PT, while the third isolate had an MIC > 256 mg/L. Using high inocula, all three isolates had an MIC > 256 mg/L. No changes in activity were seen with meropenem in relation to the bacterial inoculum used. The three isolates were susceptible to cefoxitin and amikacin (MIC, 8 mg/L).

Comments

This case exemplifies two important points: first, the possibility of therapeutic failure with PT in case of infection by ESBL-producing *K pneumoniae* and, second, the combination with amikacin does not avoid the emergence of resistance to the companion antibiotic, in this instance, ciprofloxacin. The mechanism of resistance to PT was not characterized; no mutations were identified in the gen *bla* that could explain the resistance to tazobactam, on the other hand, the susceptibility to cefoxitin ruled out the possibility of resistance due to a loss of a porin.

Mesa RJ, Blanc V, Blanch AR, et al. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage). J Antimicrob Chemother. 2006;58:211-5.

This study analysed the presence of ESBL- producing *Enterobacteriaceae* in clinical samples, in faecal samples of the population seen in The Emergancy Department, in sewage waters, in faeces of farm animals, in faeces of patients with gastroenteritis, and in samples from food. Faecal samples were cultured in a 2 mg cefotaxime containing medium. The study was carried out in 2003 and during the same period the estimated comsumption of antimicrobials in ambulatory care was measured.

The prevalence of ESBL-producing *Enterobacteriaceae* was 1.9% in clinical isolates, 6.6% in faecal samples, and 31% in samples obtained in patients with gastroenteritis. Their prevalence in samples from farm animals ranged from 20 and 100%, and in food was 0.4%. Of note, 79% of food samples analysed had been previously cooked.

The prevalence of faecal carriers was significantly higher in July to November as compared to the period of February to May. The lowest prevalence rates corresponded to the periods with highest antibiotic consumption. Given the fact that amoxicilin-clavulanate was the most frequently used antimicrobial, the authors speculate on the possibility that its use could be a reason for a lower detection of ESBL-producing strains. However, the impact of other variables such as the difference in alimentary habits (increased comsumption of of uncooked foods during the summer) should be considered.

Comments

The prevalence of ESBL-producing *Enterobacteriaceae* in this area of Spain is considerable in all different environments studied. These findings suggest that the wide dissemination of these strains in the community could be transmitted to humans via the food chain.

Vourli S, Tsorlini H, Katsifa H, Polemis M, Tzouvelekis LS, Kontodimou A, et al. Emergence of *Proteus mirabilis* carrying the *bla* metallo-beta-lactamase gene. Clin Microbiol Infect. 2006;12:691-4.

Metalo-beta-lactamase (MLB) producing *Enterobacteriaceae* are increasingly recognized in some European countries, particularly VIM derivatives. In Greece, they have been identified in *Escherichia coli*, *Enterobacter cloacae* and in *Klebsiella pneumoniae*. The last species is considered endemic in this country. Vourli et al detected (between June 2004 and March 2005) in a single institution in Greece seven *Proteus mirabilis* isolates resistant to cefotaxime, ceftazidime and imipenem, but susceptible to aztreonam. The use of the double disk diffusion assay with EDTA and imipenem was useful to ascertain the production of a MLB, unlike the E-test. Pulsed-field electrophoresis analysis revealed four related clones representing all these isolates indicating persistence of this strain in the hospital setting. In all cases, VIM-1 metallo-beta-lactamase was identified. The corresponding $bla_{\text{VIM-1}}$ gene was detected in a chromosomally encoded integron platform widely disperse in Greece and commonly associated with a plasmid. All *P. mirabilis* isolates were recovered form patients hospitalized during a prolonged period of time in three different wards, which indicate persistence over time and dispersion through the single institution.

Comments

This is the first description of a *P. mirabilis* producing VIM-1 that also illustrates the potential for dissemination of multi-resistance isolates. This article should also be considered as an alert of potential failure of the Etest strips in the detection of MLB in certain *Enterobacteriaceae*.

Gram-negative non-fermenters

Aboufaycal H, Sader HS, Rolston K, Deshpande LM, Toleman M, Bodey G, et al. $bla_{\text{VIM-2}}$ and $bla_{\text{VIM-7}}$ carbapenemase-producing *Pseudomonas aeruginosa* detected in a tertiary care medical center in the United States: report from the MYSTIC program. J Clin Microbiol. 2007;45:614-5.

Metalo-beta-lactamases (MBLs) in *Pseudomonas aeruginosa* have been mainly described in Asia and Europe and remain scarce in the USA. Aboufaycal et al. detected three multi-resistant *P. aeruginosa* isolates with a positive metallo-beta-lactamase test during a continuous surveillance study (the MYSTIC study) performed in a single institution in USA (Anderson Cancer Center) over a 7

year period (1999 to 2006). Isolates were recovered from unrelated patients and displayed different pulsed-field electrophoresis patterns as well as different ribotypes suggesting an independent emergence, possibly under carbapenem usage. Two of these isolates produced the VIM-7 enzyme, in one of them simultaneously with OXA-45, whereas VIM-2 enzyme was identified in the remaining one. In both VIM-7 producing isolates, $bla_{\text{VIM-7}}$ gene was associated with an identical integron platform, which may suggest horizontal gene transfer processes. Both patients were treated with carbapenems. Interestingly, the VIM-2 producing isolate was recovered form a patient previously treated with carbapenems in Jordan.

Comments

This study shows a well documented example of selection and spread of multi-resistant isolates from distant geographic areas, In this case, a VIM-2 producing *Pseudomonas aeruginosa*. An interesting point addressed by the authors was the phenotypic detection of MLBs in these *P. aeruginosa* isolates. In one them the double disc synergy approximation test with EDTA was negative with imipenem and in another one with meropenem. In addition, in two isolates this double disk diffusion assay was negative with ceftazidime. The use of 2-mercaptopropionic acid did not enhanced detection of MLB producing *P. aeruginosa* isolates. These results also illustrate the laboratory difficulties to detect the presence of MLBs in *P. aeruginosa*.

Falagas ME, Koletsi PK, Kopterides P, Michalopoulos A. Risks factors for isolation of strains susceptible only to polymyxin among patients with *Pseudomonas aeruginosa* bacteremia. Antimicrob Agents Chemother. 2006;50:2541-3.

Pseudomonas aeruginosa is a nosocomial pathogen with resistance to antimicrobials, both intrinsic and acquired. The rise of resistance has evolved to the appearance of epidemic caused by strains only susceptible to polymyxins, but the risk factors associated to this fact are not well known. The present study was designed to know the risk factors associated to the development of infections caused by strains of *P. aeruginosa* only susceptible to polymyxins. A retrospective case-control study was carried-out in a tertiary hospital in Athens, Greece, including all the patients with a episode of *P. aeruginosa* bacteraemia (n = 70) between January 2002 and August 2005. Cases were those with bacteraemia caused by strains only susceptible to polymyxins (n = 16) and controls those with bacteraemia caused by strains susceptible at least to polymyxins and carbapenems (n = 40). Those patients with bacteraemia caused by *P. aeruginosa* strains susceptible to polymyxins and other antimicrobials, but resistant to carbapenems, were excluded (n = 14). The most common sources of bacteraemia were pneumonia, unknown, urinary tract infections, and intra-abdominal infections. Different factors were evaluated, including the use of antimicrobials, considering only those that the patients had received during the hospitalization and at least for three days. Mortality rates were 62.5% and 37.5% in cases and controls, respectively. A multivariable logistic regression model revealed that the only factor associated to the development of infections caused by strains of *P. aeruginosa* only susceptible to polymyxins was the previous use of carbapenems (OR 9; $P = 0.001$).

Comments

This study proved the rapid emergence of resistance to carbapenems among patients infected by *P. aeruginosa* receiving this antimicrobial class. The exclusion of patients infected by strains susceptible to polymyxins but resistant to carbapenems is a major limitation of the study because the over-estimation of the use of carbapenems as a risk factor, prompted by the criteria to select the control group and to exclude patients of the study. Also, the authors do not comment if the occurrence of infections by strains only susceptible to polymyxins was related to outbreaks nor they made molecular analysis of these strains, to be sure that there was not horizontal transmission of them.

Fournier PE, Richet H. The Epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin Infect Dis. 2006;42:692-9.

The objective of this manuscript was to review the non resolved aspects of the infections caused by *Acinetobacter baumannii*. The genus *Acinetobacter* consists of strictly aerobic, gram-negative cocobacillary rods. Different studies have resulted in the description of 25 genomic species, 17 of the have been validated. In relation with the habitat, *Acinetobacter* colonize skin of human beings (< 1% *A. baumannii*); recent studies have shown *A. baumannii* in unsuspected reservoirs as foods (fruits and vegetables) and arthropods (body lice); *A. baumannii* has been the cause of infections in traumatic injuries in Iraq, Kuwait, Afghanistan, and Vietnam, suggesting environmental contamination of wounds, although the source remains unknown; also, *A. baumannii* shows tolerance to the desiccation. *A. baumannii* develops rapidly antimicrobial resistance, related to the use of antimicrobials in hospitals, through multiple mechanisms: plasmids, integrons, transposons, and natural transformation; it results in the frequent appearance of strains resistant to almost all available antimicrobials. The most frequent infections caused by *A. baumannii* affect respiratory tract, surgical wounds, urinary tract, and bacteraemia. The crude mortality is high in some infections, as bacteraemia and ventilator-associated pneumonia, although they seem have non-attributable mortality in case-control studies. The frequency of infections and colonisations by *A. baumannii* differs among hospitals, and they are mostly nosocomial; the incidence in paediatric wards is low. The outbreaks of infections by *A. baumannii* are facilitated by its resistance to the desiccation and antimicrobial resistance; they occurred in all hospital areas, being more frequent in intensive care units; multiple risks factors have been identified, including wards with high density of colonized/infected patients, environmental contamination, and carriage by hands of staff members; the adherence to strict infection-control measures is useful. The complexity of the epidemiology of *A. baumannii* infections is highlighted by the existence of centres with endemic/epidemic situations in which multiple clones are responsible of colonisations/infections; also, there is coexistence of outbreaks with sporadic cases of

infection. There has been transmission of strains among different sanitary centres in several countries. Respect to the treatment, different clinical studies have shown the efficacy of sulbactam and colistin in the treatment of cases of infections caused by multi-drug resistant strains, although there have been contradictory results in experimental models in the case of colistin.

Comments

In depth review of some selected aspects of *Acinetobacter baumannii* infections. The authors identify several fields in which we need more knowledge: virulence of *A. baumannii*, identification of reservoirs, strategies for the control of multi-drug resistant strains, and the treatment of infections caused by these strains.

Leung WS, Chu CM, Tsang KY, Lo FH, Lo KF, Ho PL. Fulminant community-acquired *Acinetobacter baumannii* pneumonia as a distinct clinical syndrome. Chest. 2006;129:102-9.

Acinetobacter baumannii is a nosocomial pathogen causing, among other infections, hospital-acquired pneumonia (HAP). Previously, there were some reports of community-acquired pneumonia (CAP) caused by *A. baumannii*. The objective of this manuscript was to analyze the clinical and prognostic characteristics of CAP by *A. baumannii* and to compare them with HAP by this bacterium. The method was a retrospective case-control study (cases: CAP; controls: HAP) carried-out in a regional hospital in Hong Kong between July 2000 and December 2003. Pneumonia was defined by clinical and radiographic criteria from the Centers for Disease Control and Prevention. *A. baumannii* was considered "definite pathogen" if isolated from blood or pleural fluid, and "probable pathogen" if isolated from sputum or tracheal aspirate cultures. Nineteen cases (CAP) and 74 controls (HAP) were analyzed. Compared with the HAP, CAP had more smokers (84 vs. 55%; $P = 0.03$) and more patients with chronic obstructive pulmonary disease (63 vs. 29%; $P = 0.01$). Also, from a clinical point of view, CAP had bacteraemia more frequently (31 vs. 0%; $P < 0.001$), as were the presence of acute respiratory distress syndrome (84 vs. 17%; $P < 0.001$), and disseminated intravascular coagulation (57 vs. 8%; $P = 0.001$). Finally, the mortality rate at 30 days, was higher in CAP than in HAP (57 vs. 35%; $P < 0.001$). Factors associated to mortality in CAP were: bacteraemia, low platelet count, acidosis, and disseminated intravascular coagulation.

Comments

These data are consistent with that from previous studies. One limitation of the results is that the number of polymicrobial pneumonias was high: 21% and 62% in CAP and HAP, respectively, resting specificity to the aetiologies of the cases and controls included in the study. Also, it would be useful to know the percentage that CAP by *A. baumannii* represents in the total of cases of CAP attended in the hospital in the period of the study. Finally, one hypothesis from the study is the possibility that only the severe cases of CAP by *A. baumannii* were admitted to the hospital.

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